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Original Article

COMT rs737865 mediates chemobrain in breast cancer patients with various levels of Ki-67

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Abstract: Previous findings have indicated that catechol-O-methyltransferase (COMT) may be a genetic risk factor for chemobrain. However, the mediation of chemobrain by COMT polymorphisms in breast cancer patients with various levels of Ki-67 remains unknown. The current research assessed the genetic risk across COMT genotypes for chemobrain in breast cancer patients with various levels of Ki-67. Breast cancer patients (65 with Ki-67<14%, 75 with Ki-67>14%) completed cognitive tests before and after adjuvant chemotherapy, and three single-nucleotide polymorphisms (SNPs) of COMT (rs165599, rs4680, rs737865) were genotyped from peripheral blood. Lower cognitive test results in breast cancer patients were displayed in those before chemotherapy. Furthermore, the event-based prospective memory (EBPM) scores of patients in the Ki-67>14% group were worse than those in the patients in the Ki-67<14% group after chemotherapy (z=-7.51, *P*<0.01), but the time-based prospective memory (TBPM) scores of the two groups were not significantly different. The COMT rs737865 A/G genotype was associated with memory protection (codominant model: adjusted odds ratio (OR)=0.135, 95% CI=0.026-0.706, *P*=0.018), and A/G genotype carriers exhibited better performance on the EBPM test than the A/A genotype. Levels of Ki-67 were likely to be associated with EBPM decline in breast cancer patients. Taken together, COMT rs737865 polymorphisms are a potential genetic risk factor for chemobrain in breast cancer patients with various levels of Ki-67.

Keywords: Catechol-O-methyltransferase (COMT), chemobrain, Ki-67, polymorphisms, prospective memory, breast cancer

Introduction

Breast cancer is one of the most common cancers in women, and 30% of new cases of cancer were breast cancer according to the American Cancer Society in 2021 [1]. Similarly, breast cancer was the primary cause of death among all cancers in women, and the estimated death rate of breast cancer was 6.9% in 2020 [2]. It was estimated that the occurrence of new cases and deaths was 303,600 and 70,400, respectively, for breast cancer in China in 2015 [3]. The incidence rate of breast cancer significantly increased in the United States from 2009 to 2018, while death rates did not increase at the same speed, indicating improvements in clinical treatment in breast cancer patients [4]. Chemotherapy is one of the main systemic therapeutics, and the survival rate of breast cancer patients has greatly improved in the past few decades [5]. Chemotherapy treatment not only prolongs the survival of breast cancer patients but also results in a number of side effects, including cognitive impairment [6]. Breast cancer patients who receive chemotherapy often complain of memory loss, poor concentration, slower processing speed, decreased word-finding skills and other cognitive impairments; these cognitive changes are collectively called chemobrain [7, 8]. Mild to severe cognitive deficits have been noted in breast cancer patients while undergoing chemotherapy [9, 10]. Approximately 75% of cancer patients during the period of chemotherapy and 35% of cancer patients experience chemobrain for several months at the end of therapy [11].

Prospective memory is a complex cognitive process involving future plans or intentions that can be separated into event-based prospective

memory (EBPM) and time-based prospective memory (TBPM) on account of the cues [12]. Results published in the journal Psychooncology indicated that EBPM impairments were found among breast cancer patients undergoing chemotherapy [13].

Chemobrain clinically presents with high levels of heterogeneity, in which some patients may have features of cognitive impairment that range from mild to severe, and some patients show no impairment [14, 15]. Breast cancer is a highly specific female tumor that needs to be diagnosed by immunohistochemistry; it is highly heterogeneous at the molecular level due to molecular genetic changes, which leads to great differences in prognosis and treatment response [16]. Ki-67 is an important indicator assessed by molecular typing and can be divided into luminal A and luminal B subtypes on account of the expression level of Ki-67 in luminal breast cancer [17]. However, the critical cutoff value for distinguishing high and low levels of Ki-67 expression has constantly changed in the literature, and the optimal value is still controversial and uncertain [18, 19]. According to the St. Gallen consensus criteria (2011 and 2013), a threshold level of 14% for Ki-67 was introduced for molecular typing in breast cancer patients and has been widely exploited in clinical practice [20, 21]. The necessary condition for luminal A was Ki-67<14%, which is associated with a better prognosis, and this value is included in most molecular typing guidelines for breast cancer screening [22, 23]. Ki-67 levels were shown to be correlated with the differentiation, proliferation and invasion, metastasis and prognosis of tumor cells [24]. It was found that learning and memory in mice was closely related to cell proliferation in the dentate gyrus by detecting levels of Ki-67 expression [25]. The expression of Ki-67 in primary pediatric brain tumors was related to poor prognosis and tumor grade [26]. Ki-67 levels were positively related to brain metastasis in lung cancer patients, and high expression indicated poor prognosis [27]. Similarly, risk factors for brain metastasis were significantly correlated with high expression levels of Ki-67 in breast cancer [28]. Our research group found that the heterogeneity in the manifestation of chemobrain is closely related to its molecular type in breast cancer patients; in other words, those with estrogen/progesterone receptor (ER/PR)-negative breast cancer were more prone to decreased EBPM performance after chemotherapy [29]. However, the relationship between chemobrain among breast cancer patients with various levels of Ki-67 is still uncertain.

The single-nucleotide polymorphism (SNP) loci of catechol-O-methyltransferase (COMT; rs4680, rs165599, and rs737865) were shown to be relevant to cognitive competence [30]. The translational product of the COMT gene plays a key role in clearing catecholamines (such as dopamine, epinephrine and norepinephrine) in the human brain [31]. Small et al. [32] found that COMT Val carriers were more likely to have poorer performance on tests of attention and verbal fluency among breast cancer patients treated with chemotherapy. Our team found that a genetic polymorphism of COMT (rs165599) was connected with retrospective memory impairments [33]. Furthermore, EBPM impairments were determined to be related to COMT rs737865 in breast cancer with different hormonal receptors [34]. However, the relationship between various levels of Ki-67 and COMT polymorphisms and chemobrain needs to be explored in breast cancer patients.

The current study concentrated on chemobrain in breast cancer survivors with various levels of Ki-67 and evaluated the genetic risk of COMT polymorphisms on chemobrain in breast cancer patients with different expression levels of Ki-67.

Materials and methods

Participants

A total of 175 women diagnosed with breast cancer were enrolled at the Department of Oncology, the Affiliated Second Hospital of Anhui Medical University. The patients were divided into groups with Ki-67<14% (99 patients) and Ki-67>14% (76 patients) [22]. The research was approved by the Research Ethics Committee of the Second Affiliated Hospital of Anhui Medical University, China. Written informed consent was obtained from all patients before the research was initiated.

All subjects met the following inclusion criteria: 1) breast cancers were diagnosed by immunohistochemistry and postoperative pathology; 2) anthracycline and paclitaxel were the main standard chemotherapy regimens, and no hormonal therapy was involved; 3) overall cognitive function was normal, and the Mini-Mental State Examination (MMSE) scores were greater than 24; 4) activities of daily living were normal, and Karnofsky performance scale (KPS) scores were ≥80 points; and 5) no communication barriers were present. The exclusion criteria were as follows: 1) a history of radiotherapy and endocrine therapy; 2) terminal stage of tumor; 3) brain metastases; 4) mental illness, such as dementia; 5) a history of antipsychotics and psychotherapy; and 6) severe anxiety and depression.

Neuropsychological tests

A series of neuropsychological tests were performed before and after chemotherapy.

The specific time points for cognitive testing were one week before the initiation of chemotherapy and one month after the six standard chemotherapy sessions. The MMSE was used to assess general cognitive function, including the following seven aspects: orientation in time and place, immediate memory, attention and concentration, delayed memory, language and visual-spatial skills. For each of the 30 questions, 1 point was given for a correct answer, and 0 points were given for a wrong answer or the answer was unknown. A score of 26 or less on the MMSE indicated cognitive impairment. Language competence, semantic memory and executive function were evaluated by the verbal fluency test (VFT). The test required the participants to name as many words of a certain category as possible in a given time (usually one minute). One point was given for each answer. The digit span test (DST) was mainly applied to assess the attention and immediate memory of the subjects; for this test, the highest score corresponding to the forward and backward recall of numbers was recorded.

Event-based prospective memory (EBPM) task

The subjects were told to tap the table whenever they saw two animal words (target words) in the EBPM task. Furthermore, the subjects were asked to remember to state their contact number (no additional prompts were given) at the end of the experiment. There were 32 Chinese cards, each of which contained 12 Chinese words: two of 12 words belonged to a subcategory and the other 10 words belonged to a major category. The patient's task was to select the two words belonging to the subcategory. When the selected words were target words (animal category), the subjects were required to knock on the table. A total of 6 target words were interspersed among the trials. If the subjects remembered to complete the task correctly, one point was recorded for each word for a total of 6 points. At the end of the task, the subject was instructed to say his or her contact number, and if this was correctly remembered, 2 points were given. The total possible EBPM score was 8 points.

Time-based prospective memory (TBPM) task

The subjects were required to occasionally perform a target behavior, and the number of times that the target behavior was correctly executed was taken as the score of the TBPM task. There were 100 cards in total, of which 12 cards had two-digit numbers on them. The subject's task was to select the maximum and minimum numbers on each card and were asked to knock on the table every five minutes after the start of the selection task. The time could be monitored through a clock placed at the right shoulder of subjects, and the total test time was 17 min. If the subjects could remember to tap the table in the interval from 10 s before to 10 s after the target time, 2 points were given, and 1 point was given if the tapping on the table occurred in the interval from 30 s before to 30 s after the target time. The total possible TBPM score was 6 points.

Genotyping

Peripheral venous blood (3~5 ml) was collected from each participant, put into a vacuum blood collection tube, and stored in a refrigerator at -20°C. Genomic DNA was extracted by a blood genomic DNA QIAGENE Kit (Shanghai Genesky Bio-Tech Co., Ltd.) (http://biotech.geneskies.com). Genotyping was accomplished by Shanghai Genesky Biotechnology Co., Ltd., adopting the technology of the improved multi-

Table 1. The basic clinical characteristics of breast cancer patients with various index of Ki-67

Contents		Groups	
		Ki-67<14% (n=99)	Ki-67>14% (n=76)
Age (Mean ± SD, year)		49.04±9.97	48.13±10.29
Education (Mean ± SD, year)		10.15±3.85	10.05±3.30
KPS (Mean ± SD, year)		85.25±6.90	82.37±7.81*
Pathological patterns (%)	IDC-NOS	89 (89.9%)	71 (93.4%)
	IDC-S	1 (1.0%)	3 (4.0%)
	CIS	9 (9.1%)	2 (2.6%)
Stages (%)	I	8 (8.1%)	2 (2.6%)**
	II	41 (41.4%)	52 (68.4%)
	III	16 (16.2%)	9 (11.8%)
	IV	33 (34.3%)	13 (17.1%)

Note: *: $P<0.05$, **: $p<0.01$. KPS, karnofsky performance status scale; IDC-NOS, non-special type invasive ductal carcinoma of breast; IDC-S, special type invasive ductal carcinoma of breast; CIS, carcinoma in situ; MIC, microinvasive carcinoma.

plex ligase detection reaction (iMLDR). The three SNP loci of 175 samples were genotyped in this study. The region of the target SNP loci was amplified in one system using multiplex PCR. The amplified products were purified by exonuclease and used as templates for subsequent ligase reactions. The 5'-end allele-specific probes and a fluorescently labeled specific probe at the 3'-end were contained in each site for the linkage reaction. The ligase products were amplified by PCR using fluorescently labeled universal primers. The amplified products were distinguished by fluorescence capillary electrophoresis. Finally, the genotypes of each SNP locus were acquired by electrophoresis analysis. The success rate of classification was 99%, and the accuracy was 99.6%.

Statistical analysis

The SPSS software package (version 22.0, http://spss.en.softonic.com/; Chicago, IL, USA) was applied to conduct the statistical analysis. Forest plots and histograms were drawn with GraphPad Prism 5 (Graph Pad Software Inc., San Diego, CA). The clinical baseline characteristics and cognitive tests were compared across the Ki-67<14% group and the Ki-67>14% group, adopting independent-sample t tests or Mann-Whitney U tests for normally distributed data and nonnormally distributed data, respectively. The genetic stability in the population was detected passing Hardy-Weinberg equilibrium (HWE). The differences in allelic genes, genotype frequency, pathological type and neoplasm staging were analyzed by the chi-square (X^2) test in the two groups. Binary logistic regression was performed to assess genetic susceptibility risk factors associated with chemobrain, which were displayed as odds ratios (ORs) and 95% confidence intervals (CIs).

Genetic models were developed to further evaluate the susceptibility factors causing cognitive dysfunction, including codominant, dominant, recessive, HOM and HET models. The *P* value of the logistic regression was adjusted for age, KPS score, education, neoplasm staging, and pathological pattern. One-way ANOVA was applied to analyze the cognitive impairment across different genotypes among breast cancer patients with Ki-67>14%. All statistical tests were two-tailed with a statistical significance criterion defined at $P<0.05$.

Results

Clinical characteristics

As shown in **Table 1**, the Ki<14% group included 99 patients, and the Ki-67>14% group included 76 patients. There were significant differences in the KPS scores (85.25±6.90 vs. 82.37±7.81, respectively; z=-2.53, $P<0.05$). However, age (49.04±9.97 vs. 48.13±10.29) and years of education (10.15±3.85 vs. 10.05±3.30) were not significantly different. Similarly, there was a significant difference in tumor stages between the two groups ($\chi^2=13.84$, $P<0.01$). However, pathological patterns were not significantly different. In the Ki-67<14% group, 89 breast cancer patients were confirmed to have nonspecial-type invasive ductal carcinoma (IDO-NOS), 1 patient was confirmed to have special-type invasive ductal carcinoma (IDO-S), and 9 patients were confirmed to have carcinoma in situ (CIS). Similarly, in the Ki-67>14% group, 71 breast cancer patients were confirmed to have IDO-NOS, 3 patients were identified as having IDO-S, and 2 patients were confirmed to have CIS.

Table 2. General cognitive test before and after chemotherapy

Task	Mean ± SD	
	Before chemotherapy (n=175)	After chemotherapy (n=175)
MMSE	27.27±1.54	26.64±1.70**
DST	6.18±0.69	5.78±0.98**
VFT	11.35±1.54	9.81±2.08**
EBPM	2.67±1.01	1.82±1.18**
TBPM	4.99±0.98	4.71±0.92**

Note: **: *P*<0.01. MMSE indicates the mini-mental state; DST indicates the digit span test; VFT indicates the verbal fluency test. EBPM indicates the event-based prospective memory; TBPM indicates the time-based prospective memory; RM retrospective memory; PM prospective memory.

Table 3. Cognitive test with various index Ki-67 groups after chemotherapy

Task	Groups (Mean ± SD)	
	Ki-67<14% (n=99)	Ki-67>14% (n=76)
MMSE	26.93±1.49	26.26±1.88*
DST	6.02±0.85	5.48±1.07**
VFT	10.38±1.99	9.05±1.96**
EBPM	2.37±1.08	1.09±0.85**
TBPM	4.68±0.99	4.75±0.80#

Note: #: *P*>0.05, *: *P*<0.05, **: *P*<0.01. MMSE: mini-mental state; DST: digit span test; VFT: verbal fluency test; EBPM: event-based prospective memory; TBPM: time-based prospective memory.

Table 4. Information for 3 genotyped SNPs of COMT in various index ki-67 groups

SNP	COMT		
	rs4680	rs165599	rs737865
CHR	22	22	22
Allele Position	19951271	19956781	19930121
Allele type	G/A	G/A	A/G
MAF	0.255	0.500	0.307
P for HWE	0.318	1	1
*P**	0.931	0.197	0.037*

Note: *: *P*<0.05, Single nucleotide polymorphism (SNP); Chromosome (CHR); Minor allele frequency (MAF, data from 1000 Genomes); Hardy-Weinberg equilibrium (HWE), *p*-value for HWE in 2 groups; **p*-value for allelic frequency differences between two groups.

General cognitive testing before and after chemotherapy

Table 2 shows that before and after chemotherapy, the MMSE (27.27±1.54 vs. 26.64±1.70), DST (6.18±0.69 vs. 5.78±0.98), VFT (11.35±1.54 vs. 9.81±2.08), EBPM (2.67±1.01 vs. 1.82±1.18) and TBPM (4.99±0.98 vs. 4.71±0.92) scores significantly decreased (z=-3.07, z=-3.58, z=-6.95, z=-6.72, z=-2.77, respectively, *P*<0.01).

General cognitive testing after chemotherapy

Table 3 shows that the TBPM scores of breast cancer patients in the Ki-67<14% group after chemotherapy were slightly higher than those in the Ki-67>14% group (4.68±0.99 vs. 4.75±0.80), although there was no significant difference (*P*>0.05). In contrast, the MMSE scores were higher in the Ki-67<14% group after chemotherapy than in the Ki-67>14% group (26.93±1.49 vs. 26.26±1.88, *P*<0.05). Similarly, the DST, VFT, and EBPM scores were significantly different in the Ki-67<14% and Ki-67>14% groups after chemotherapy (DST: 6.02±0.85 vs. 5.48±1.07; VFT: 10.38±1.99 vs. 9.05±1.96; EBPM: 2.37±1.08 vs. 1.09±0.85, *P*<0.01).

Sequencing analysis

Table 4 indicates that the allelic distribution of COMT rs737865 was significantly different between the Ki-67<14% and Ki-67>14% groups (*P*=0.037). The COMT SNPs (rs4680, rs165599, rs737865) all conformed to HWE in the 2 groups (P>0.05). This revealed that the three SNP loci we chose in our study were genetically stable, and group representation was discovered in the Ki-67<14% and Ki-67>14% groups.

As shown in **Table 5**, there was a significant difference in the rs737865 (recessive model: χ^2=5.156, *P*=0.025) genotypic frequency distribution. Furthermore, as shown in **Table 6** and **Figure 1**, binary logistic regression analysis results revealed that the patients with the A/G (adjusted OR=0.135, 95% CI=0.026-0.706, *P*=0.018) genotype of COMT rs737865 had a significantly lower risk of developing cognitive impairment than the patients with the A/A genotype. Regarding the genetic models, the recessive model and HOM model of rs737865 with the G/G genotype (adjusted OR=0.162, 95% CI=0.032-0.818, *P*=0.028; adjusted OR=0.123, 95% CI=0.022-0.680, *P*=0.016, respec-

Table 5. Genotype frequencies of COMT (rs4680, rs165599, rs737865) in various index ki-67 groups

SNP	Model	Genotype	Ki-67<14%	Ki-67>14%	χ^2	P
rs4680	Co-dominant	G/G	56	44		
		G/A	36	25	0.408	0.819
		A/A	7	7		
	Dominant	G/A+A/A	43	32	0.031	0.879
		G/G	56	44		
	Recessive	A/A	7	7	0.267	0.780
		G/G+G/A	92	69		
rs165599	Co-dominant	G/G	24	23	1.765	
		G/A	49	39		0.400
		A/A	26	14		
	Dominant	G/A+A/A	75	53	0.793	0.394
		G/G	24	23		
	Recessive	A/A	26	14	1.499	0.276
		G/G+G/A	73	62		
rs737865	Co-dominant	A/A	46	43	5.747	
		A/G	41	30		0.052
		G/G	12	2		
	Dominant	A/G+G/G	53	32	2.017	0.171
		A/A	46	43		
	Recessive	G/G	12	2	5.156	0.025*
		A/A+A/G	87	73		

Note: *: $P<0.05$, The χ^2 test of P values for SNP polymorphisms distribution differences between ki-67<14% and ki-67>14% group; Models: Various genetic models that were defined as 1 (MM+Mm) versus 0 (mm) for dominant; 1 (mm) versus 0 (MM+Mm) for recessive; and 0 (mm) versus 1 (Mm) versus 2 (MM) for co-dominant (M and m represent major and minor alleles, respectively).

tively) decreased the risk of experiencing chemobrain. There was no statistically significant difference in the loci of COMT rs4680 and rs165599 between the Ki-67<14% and Ki-67>14% groups.

Correlation analyses between COMT rs737865 polymorphisms and chemobrain

Means and standard deviations for the neuropsychological test results are shown in **Table 7** and **Figure 2**. The A/G and G/G genotype carriers of COMT rs737865 had higher EBPM scores than A/A carriers (1.31±0.82 vs. 0.91±0.84, respectively, $P<0.05$). Similarly, the A/G genotype carriers of COMT rs737865 presented significantly higher EBPM scores than A/A carriers (1.33±0.80 vs. 0.91±0.84, respectively, $P<0.05$) in the Ki-67>14% group of breast cancer patients.

Discussion

Through the detection of COMT gene polymorphisms in peripheral blood and the evaluation of the MMSE, DST, VFT, EBPM and TBPM, the results showed that 1) the EBPM and TBPM deficits were present in breast cancer following chemotherapy; 2) breast cancer patients with Ki-67>14% had worse results than patients with Ki-67<14% on the MMSE, DST, VFT and EBPM tasks after chemotherapy; and 3) there were genotypic differences in COMT rs737865 between the Ki-67<14% and Ki-67>14% groups, the A/G genotype was associated with memory protection, A/G genotype carriers exhibited better results on the EBPM test than the A/A genotype, and the COMT rs737865 polymorphism could be a potential genetic risk factor associated with chemobrain in breast cancer patients with various levels of Ki-67.

Cognitive function in animals can be affected by single or combined chemotherapy, and the learning and memory functions related to the hippocampus are impaired after chemotherapy [35]. Doxorubicin is a commonly used chemotherapeutic drug for breast cancer, and patients treated with doxorubicin had poor scores on

Table 6. Genetic susceptibility of COMT (rs4680, rs165599, rs737865) gene in various index ki-67 groups

SNP	Model	Genotype	Ki-67<14%	Ki-67>14%	Binary logistic regression	
					OR (95% CI)	P
rs4680	Co-dominant	G/G	56	44	-	-
		G/A	36	25	1.318 (0.415-4.186)	0.639
		A/A	7	7	1.477 (0.445-4.905)	0.524
	Dominant	G/A+A/A	43	32	0.964 (0.508-1.831)	0.912
		G/G	56	44		
	Recessive	A/A	7	7	1.378 (0.448-4.242)	0.572
		G/G+G/A	92	69		
	HOM	-	-	-	1.106 (0.347-3.523)	0.865
	HET	-	-	-	0.912 (0.459-1.815)	0.794
rs165599	Co-dominant	G/G	24	23	-	-
		G/A	49	39	0.553 (0.217-1.408)	0.214
		A/A	26	14	0.604 (0.265-1.376)	0.230
	Dominant	G/A+A/A	75	53	0.788 (0.381-1.630)	0.520
		G/G	24	23		
	Recessive	A/A	26	14	0.586 (0.268-1.283)	0.181
		G/G+G/A	73	62		
	HOM	-	-	-	1.014 (0.969-1.061)	0.553
	HET				1.010 (0.974-1.046)	0.595
rs737865	Co-dominant	A/A	46	43		
		A/G	41	30	0.135 (0.026-0.706)	0.018*
		G/G	12	2	0.198 (0.038-1.036)	0.055
	Dominant	A/G+G/G	53	32	0.562 (0.294-1.074)	0.081
		A/A	46	43		
	Recessive	G/G	12	2	0.162 (0.032-0.818)	0.028*
		A/A+A/G	87	73		
	HOM	-	-	-	0.123 (0.022-0.680)	0.016*
	HET				0.681 (0.348-1.335)	0.264

Note: *: *P*<0.05, *P* value for binary logistic regression analysis; odds ratio (the OR); 95% confidence interval (95% CI); co-dominant model were defined as 0 (mm) versus 1 (Mm) versus 2 (MM); Dominant models were defined as 1 (MM+Mm) versus 0 (mm); recessive models were defined as 1 (mm) versus 0 (MM+Mm); Homozygote (HOM) were defined as 1 (MM) versus 0 (mm); and Heterozygote (HET) were defined as 1 (Mm) versus 0 (mm) (M and m represent major and minor alleles, respectively).

cognitive and visuospatial skills tests [36]. Koppelmans et al. [37] conducted cognitive testing in breast cancer patients and found that immediate and delayed verbal memory, processing speed, executive function and psychomotor speed were significantly lower than those in the controls, even after the end of treatment. Janelsins et al. [38] found in a longitudinal study that cognitive impairment could partially recover after 6 months of chemotherapy in breast cancer patients, but it did not return to the levels observed before chemotherapy. Different degrees of EBPM disruption exist in breast cancer patients following chemotherapy, and hormone receptors were shown to be related to chemobrain; specifically, individuals with ER-/PR- breast cancer had worse cognitive function [29]. In the present study, the findings indicated that patients with Ki-67>14% breast cancer suffered worse chemotherapy-related EBPM deficits than those with Ki-67<14% breast cancer.

Ki-67, a large molecule antigen located in the nucleus, is expressed in all cell cycles except G0 phase and has been identified as a highly efficient molecular marker for cell proliferation [39]. The marker Ki-67 is closely related to the

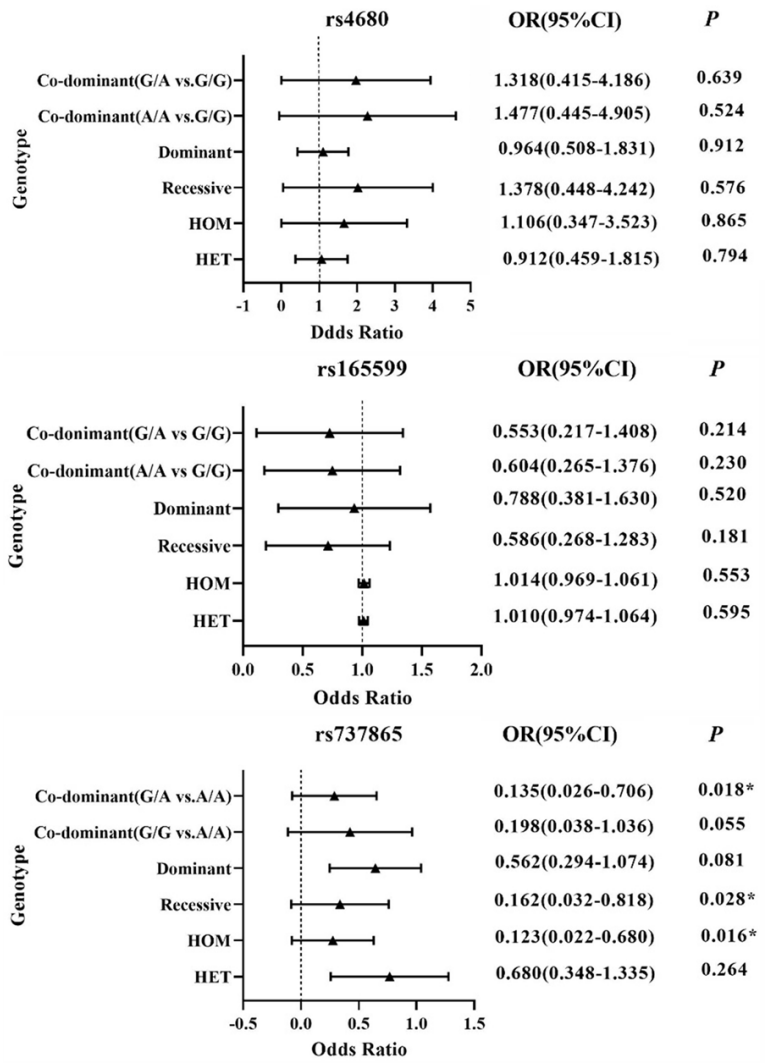

Figure 1. Forest plots of susceptibility analysis of COMT (rs4680, rs165599, rs737865) in the Ki-67<14% and Ki-67>14% groups. *P* values for logistic regression analysis adjusted for age, years of education, KPS scores, pathological patterns and tumor stages (*P<0.05).

degree of malignancy, which possesses important reference value for predicting the prognosis of tumors and has become a routine item in the pathological examination of breast cancer [40]. Higher levels of Ki-67 are associated with pathological diagnosis for primary central system tumors, with nerve numbness, cognitive deficits, disturbance of consciousness and other neuropsychiatric symptoms [41]. Minisini et al. [42] found that the risk of developing brain metastases was higher in breast cancer patients with high Ki-67, which is associated with a worse prognosis and cognitive decline. A moderate Ki-67 level was found to be significantly related to positive concentration performance in a cognitive task before adjuvant treatment [43]. The study found that breast cancer patients with high expression levels of Ki-67 were more sensitive to chemotherapy [44, 45]. Meanwhile, chemotherapy could lead to cognitive dysfunction. We demonstrate for the first time that the chemobrain is associated with Ki-67, a marker of the molecular type of breast cancer. Ki-67 was confirmed as a risk factor for chemobrain (EBPM impairment) in this study.

The three SNPs of COMT (rs4680, rs737865 and rs165599) have been common sites in the study of psychiatric disorders [46, 47]. Scores for cognitive functions such as memory, attention and executive control among Val carriers of COMT were significantly lower than those among Met carriers in schizophrenia patients [48]. Juarez-Cedillo et al. [49] found that COMT polymorphisms were associated with dementia susceptibility and cognitive impairment when investigating elderly individuals in the community. This study found that COMT polymorphisms among breast cancer patients with different Ki-67 levels were related to chemobrain. The following are possible explanations from several perspectives. First, the decline in memory ability in breast cancer patients might be related to a decrease in enzyme activity in the prefrontal cortex and hippocampus, which is caused by COMT gene polymorphisms, thus affecting the concentration of dopamine in the brain [49]. We hypothesized that breast cancer patients with a high Ki-67 levels may be more prone to COMT gene polymorphisms with reduced enzyme activity, thus influencing cognitive function. Second, COMT is widely distributed in the hippocampus and can directly regulate the level of dopamine in the hippocampus and subsequently affect hippocampal structure [50, 51]. These changes in hippocampal formation

Table 7. Comparison for cognitive test with different COMT (rs737865) genotypes

rs737865	A/G+G/G VS. A/A		G/G VS. A/A+A/G		G/G VS. A/A		A/G VS. A/A	
MMSE	26.66±1.45	25.91±2.08	27.00±1.41	26.72±1.88	27.00±1.41	25.91±2.08	26.63±1.47	25.91±2.08
DST	5.56±1.10	5.42±1.06	5.50±1.41	5.48±1.08	5.50±1.41	5.42±1.06	5.57±1.10	5.42±1.06
VFT	9.00±1.88	9.07±2.05	8.00±2.83	9.07±1.96	8.00±2.83	9.07±2.05	9.07±1.85	9.07±2.05
EBPM	1.31±0.82	0.91±0.84*	1.00±1.41	1.08±0.85	1.00±1.41	0.91±0.84	1.33±0.80	0.91±0.84*
TBPM	4.78±0.79	4.72±0.83	5.00±1.41	4.74±0.80	5.00±1.41	4.72±0.83	4.77±0.77	4.72±0.83

Note: *P<0.05. MMSE: mini-mental state; DST: digit span test; VFT: verbal fluency test; EBPM: event-based prospective memory; TBPM: time-based prospective memory.

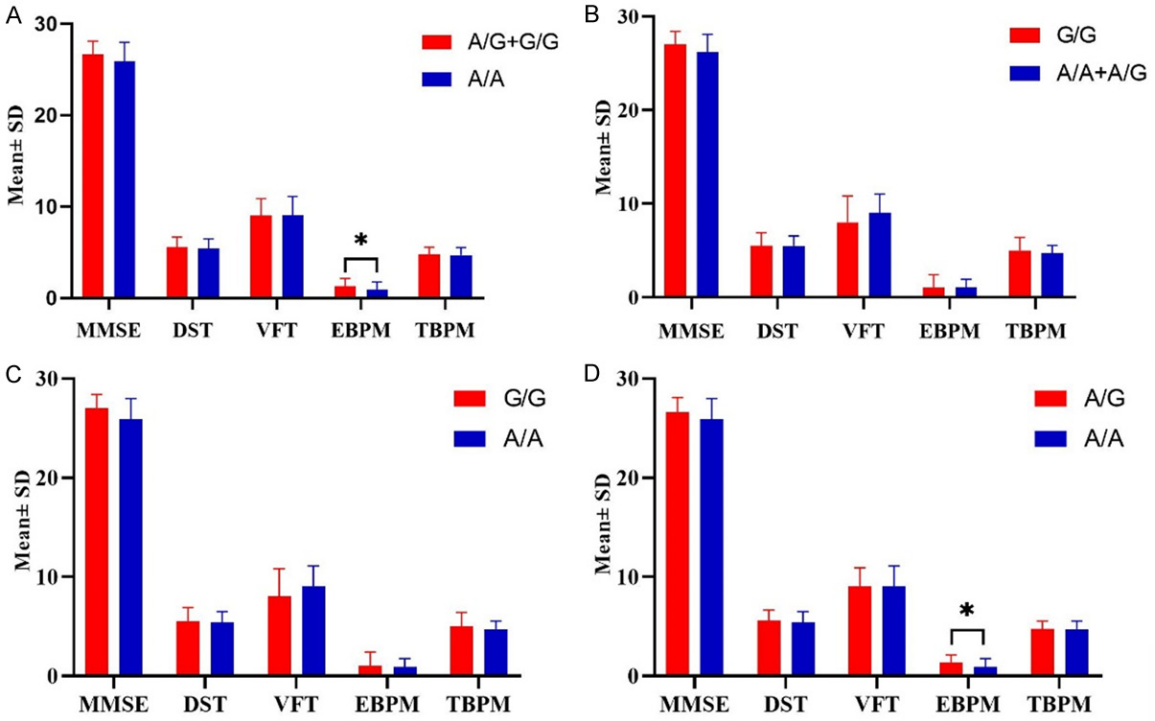

Figure 2. Histograms of cognitive test results across different COMT (rs737865) genotypes (*P<0.05). A. Comparison of cognitive scores between the A/G+G/G group and the A/A group. B. Comparison of cognitive scores between the G/G group and the A/G+A/A group. C. Comparison of cognitive scores between the G/G group and the A/A group. D. Comparison of cognitive scores between the A/G group and the A/A group.

and function could lead to a series of cognitive changes related to memory, which may explain the experience of chemobrain in breast cancer patients [52, 53]. COMT polymorphisms were shown to have a significant effect on the volume of the CA1 region in the right hippocampus [54]. Stefan et al. found that COMT gene polymorphisms were related to the volume of the medial temporal lobe [55]. Our group found that chemobrain among individuals with different molecular types (ER/PR) of breast cancer was associated with brain changes in the dorsolateral prefrontal cortex [56]. Breast cancer patients with high Ki-67 levels were potentially more prone to COMT gene polymorphisms that alter hippocampal structure and function. Finally, the level of estrogen in breast cancer patients might affect the level of COMT gene polymorphism. McDermott et al. [57] indicated that COMT expression was significantly decreased by high levels of estrogen, which influenced concentrations of catecholamines in the hippocampus and enhanced the formation of fear memories. Our past research showed that COMT rs165599 was a genetic risk factor influencing chemobrain in triple-negative breast cancer patients [33]. Chemobrain is closely related to its molecular type in breast cancer patients. What we found in this study, consistent with our hypothesis, was that the A/G gen-

otype of COMT rs737865 was a better predictor of performance on the TBPM task following chemotherapy, as it was a genetic risk factor for chemobrain in breast cancer patients with various levels of Ki-67. This was the first discovery that the COMT rs737865 A/G genotype was associated with memory protection in breast cancer patients with various levels of Ki-67.

The strength of this study was that it integrated cognitive neuropsychology, genetics and oncology to explore the mechanism of chemotherapy in the brain and it included memory scale testing, genetic detection, and chemotherapy regimen selection. However, the limitations and challenges of this study should also be stated. First, this study had only a before-and-after comparison and did not include a healthy control group, and therefore, the expression of COMT and cognitive testing in healthy women could not be used in the comparison. Second, this study focused on early cognitive impairments that occurred one month after chemotherapy. It is unknown whether the results are suitable for follow-up cognitive impairment research. Third, the sample size was insufficient, and the number of breast cancer patients and healthy controls needs to be higher in subsequent studies. Fourth, the tasks, EBPM and TBPM, were chosen based on Mcdougal's research methods. These tasks involve subjective memory representations, and cognitive scales involving objective measures for cancer patients may be better.

Conclusion

In summary, our study was the first to find a relationship between chemotherapy-related EBPM impairments and genetic polymorphisms in breast cancer patients with various levels of Ki-67. The heterogeneity in chemobrain presentation may be mediated through COMT rs737865 polymorphisms, and this mediation may indicate that COMT polymorphisms are potential genetic risk factors for chemobrain in breast cancer patients with various levels of Ki-67.

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Disclosure of conflict of interest

None.

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